



This Standard Operating Procedure (SOP) is applicable to all UniSQ Research Workers who care for and use Animals for Scientific Purposes. The procedure must only be performed by those persons who have been deemed competent, and who believe they remain competent to do so. Access to supervision by suitably qualified staff whilst undertaking this procedure is encouraged, where required.

Species

- Small mammals (for some of these the genus is also its common name)
 - *Mus musculus* (the House mouse)
 - *Antechinus* (over a dozen species of Antechinus, e.g. Agile antechinus, Brown antechinus, Yellow-footed antechinus)
 - *Pseudomys* (over 20 species of native Australian mice e.g. the New Holland mouse, Eastern chestnut mouse, Hasting River mouse)
 - *Sminthopsis* (about 19 species of Dunnarts, e.g. Fat-tailed dunnart, Julia Creek dunnart)
 - *Rattus* (includes the introduced rats in Australia – the Black and Brown rats, and the native Bush and Swamp rats)
 - *Planigale* (five species of Planigales, e.g. Common planigale, Long-tailed planigale)
 - *Phascogale* (three species of Phascogales, sometimes called wambengers, e.g. Brush-tailed phascogale, Red-tailed phascogale)

Purpose

The purpose of this SOP is to provide information to researchers/ teachers considering ear notching small mammals as part of surveying and research on wildlife, specifically small mammal populations; why small mammals are ear notched and how to ear notch them.

Population surveys and monitoring play a key component to many ecological research projects. Population data is obtained through ecological survey techniques such as camera, cage, Elliott and pitfall trapping. To obtain information about abundance from surveys, to determine population sizes (and changes) and demographic information (e.g. different age classes) it is vital to obtain information on large numbers of individual animals to obtain sufficient data to obtain accurate and statistically meaningful results.

Ear notching is the most commonly used, permanent method to individually identify small mammals, both in captive colonies and for free-living wild small mammals.

Definitions

Elliott trap	An aluminium box trap typically 30 x 8 x 10 cm with a trap door at one end. Used in the field with bait (peanut paste, oats, etc) to capture small mammals.
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Linked SOPs

SOP ID number	SOP title
WL012	Dry pitfall trapping for vertebrates
WL007	Removing small mammals from pitfall traps
WL019	Use of box and cage traps

Potential hazard to Research Workers

UniSQ Risk Management Plan ID number	UniSQ Management Plan title
RMP_2020_4960	Wildlife research and teaching fieldwork

Personal Protective equipment required

- Disposable examination gloves of various sizes

Animal wellbeing considerations

Perceived stressors	Management strategy
Stress from prolonged process	Handlers must be sufficiently trained prior to starting work on small mammals so that the process is efficient and performed with a minimum of handling.
Disease risk	Handlers must wash hands thoroughly before and after handling animals and put on a clean pair of disposable examination gloves between each animal.
Disease risk	Ear hole punch/notcher is sterilised with 70% alcohol or alcohol swab before use and between each animal.
Unwell animal	Ear notching must not be undertaken if the animal is unwell, or injured in any way. Unwell or injured animals must be monitored and assessed as per the approved animal score sheet for that species to determine what action is to be taken, i.e. Emergency euthanasia.
Heat or cold stress	Ear notching should not be undertaken if animal is likely to be exposed to temperature extremes.

The overall perceived level of risk to an animal undergoing this procedure is:

High

Medium

Low

Substances to be administered

Substance	Dose	Route	Purpose
Not applicable			

Equipment/ materials required

- Data record sheet
- Ear hole punch or notcher (1 mm for mice-sized animals; 2 mm for animals such as Brushtail possums)
- 70% ethanol and alcohol wipes
- Calico bags approximately 20 cm by 30 cm in size (double stitched with no loose threads on the inside and outside) with cloth tape or string to close the bag - if the animal has been removed from a trap, e.g. pitfall or small mammal box trap, Elliott trap.
- Microcentrifuge tubes' (1.5/2 ul) containing 70% ethanol for ear sample for DNA analysis
- Tweezers

Site specification or location requirements

At locations/ fields outlined in UniSQ AEC approved applications that include the use of this SOP.

Waste disposal

Nil.

Duration of the procedure

Procedure	1 minute
Post-procedure and monitoring	3 minutes

Procedure

Small mammal in an open container

1. Clean the ear notching instrument and tweezers with alcohol wipes.
2. If ear notch sample is to be used for genetic analysis, then label microcentrifuge tube containing 70% ethanol with species, individual ID (or trap number) and date.
3. In one movement, swiftly place the palm of your hand over the small mammal.
4. Scoop and hold the small mammal in your palm.
5. Place the forefinger of the same hand under the chin to hold the head upwards.
6. Use the other hand to position the ear for notching (Figure 1) ensuring you have determined the placement of the ear notch to match the individual number you wish to allocate to that animal (Figure 2).
7. Position ear hole punch/notcher on either side of the ear, taking care to ensure you don't cut any large blood vessels in the ear, and in one swift movement, press down.
8. Ensure that the ear is cut completely through so that the cut piece of skin is totally removed from the ear. If a tissue sample is to be kept for research purposes (e.g. genetic analysis) then carefully use disinfected tweezers to place the tissue sample into the labelled microcentrifuge tube containing ethanol. Transport samples in microcentrifuge tubes in a cool box and then store them in a refrigerator in a clearly labelled container until analysis.
9. Place the animal back into the container.
10. Check the animal 3 minutes later to check that it is alert and any bleeding from the notch has stopped. Although extremely unlikely if the animal is showing signs of distress e.g. extremely agitated, or with obvious bleeding from the ear notch seek immediate veterinary advice. Animals that die as a result of being ear notched or during subsequent handling must be reported to the UniSQ AEC.

Small mammal in a small cloth bag (i.e. calico)

1. Clean the ear notching instrument and tweezers with alcohol wipes.
2. If an ear notch sample is to be used for genetic analysis, then label microcentrifuge tube containing 70% ethanol with species, individual ID (or trap number) and date.
3. Undo the tapes or string that has closed the cloth bag while holding the small mammal through the cloth of the bag such that you are holding the small mammal in the palm of your hand.
4. While the animal is still in the calico bag, gently manipulate the small mammal such that the animal is held with one hand through the calico bag. To do this, cup the animal through the calico bag such that it is held in the palm of your hand with the head pointing towards your fingers. The thumb and second (middle) finger can be utilised to restrain the animals head (fingers on either side of the head), and the index finger is placed on the top of the head. This is the three-finger hold.
5. Holding the mammal firmly drawback the cloth such that the animal's head and ears are exposed, i.e. outside the cloth bag, while you still hold the body of the animal through the cloth back, in your palm.
6. Place the forefinger of the same hand under the chin to hold the head upwards.
7. Use the other hand to position the ear for notching (Figure 1) ensuring you have determined the placement of the ear notch to match the individual number you wish to allocate to that animal (Figure 2).
8. Position ear hole punch/ notcher on either side of the ear, taking care to ensure you don't cut any large blood vessels in the ear, and in one swift movement, press down. There should not be more than three ear notches per ear.
9. Ensure that the ear is cut completely through so that the cut piece of skin is totally removed from the ear. If a tissue sample is to be kept for research purposes (e.g. genetic analysis), then carefully use disinfected tweezers to place the tissue sample into the labelled microcentrifuge tube containing ethanol. Transport samples in microcentrifuge tubes in a cool box and then store them in a refrigerator in a clearly labelled container until analysis.
10. If necessary, to retain the small mammal in the cloth bag, pull the cloth bag up such that the animal is fully covered and close the entrance to the bag using the tape or string while holding onto the animal in your palm.
11. Tie off the entrance of the bag, so the mammal is enclosed and safe within the cloth bag.
12. If an animal is in a cloth bag it will be released back into the wild after it has been kept in the bag for three minutes. It should be released in the vicinity of where it was captured in vegetation that would offer safe refuge to the small mammal. Untie the calico bag and release the animal into that vegetation. Being a small mammal, the animal will normally disappear into the vegetation very quickly once it is released. Observe the animal until it has moved into the vegetation and if there appears to be a problem, e.g. unstable movement, record this and any other observations, e.g. missing the second toe on the left hind leg in the column labelled 'Remarks'. Animals that die as a result of being ear notched or during subsequent handling must be reported to the UniSQ AEC.



Figure 1: Hold the small mammal firmly and position the ear so it is ready for ear notching.

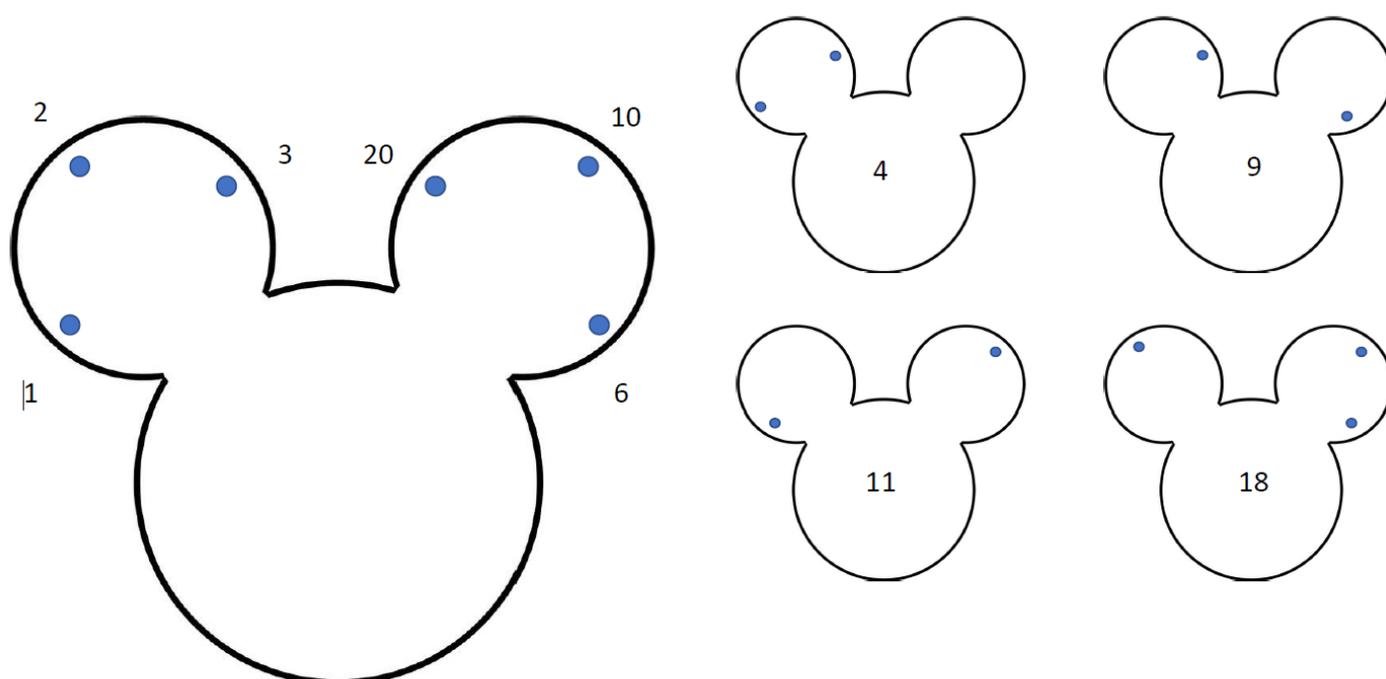


Figure 2: Ear notch location on ears used to individually identify animals, indicating that up to 3 holes can be used for individual numbers for each animal that is ear notched.

Training, qualifications or competencies required

Researchers with relevant experience or qualification can only undertake this SOP to complete the procedures required.

Student researchers must receive appropriate training and supervision from UniSQ research supervisors or qualified individuals prior to undertaking procedures.

References

Dahlborn, K., Bugnon, P., Nevalainen, T., Raspa, M., Verboost, P. & Spangenberg, E. (2013). Working Party Report; Report of the Federation of European Laboratory Animal Science Associations Working Group on animal identification. *Laboratory Animals*; 47: 2–11. DOI: 10.1177/002367712473290

Hau, J. & VanHoosier, G.L. Jr. (eds) (2002). *Methods of Identification in Handbook of Laboratory Animal Science* 2nd Edition; Vol I. Essential Principles and Practices, CRC Press, pp. 363-365.

Weyand, M.E. (1998). Methods for the Identification of Laboratory Mice and Rats. *Lab Animal*, 27:47-50.

The University of Queensland Standard operating procedures. SOP No: AHT27(d) Ear Notching in Rodents (May 2015).

Licences and permits

Any required licences and/or permits to undertake the procedure(s) under this SOP must be obtained before undertaking this SOP.

SOP approval and review history			
Date	Version	Review Pathway	Notes
17/12/2020	0.0	3/12/2020 UniSQ AEC "Subject to Modifications". 17/12/2020 Reviewed and approved by the UniSQ AEC Executive.	Approved
23/06/2021	0.1	23/06/2021 Inserted under "Licences and Permits", the words: "Any required licences and/or permits to undertake the procedure(s) under this SOP must be obtained before undertaking this SOP."	Update
18/10/2022	0.2	16/09/2022 Converted SOP to new UniSQ branding and revised 'USQ' to 'UniSQ'	UniSQ 2022 Rebrand
24/08/2023	1.0	03/08/2023 UniSQ AEC "Subject to Modifications" with the revised SOP to be reviewed by the UniSQ AEC Executive. 24/08/2023 Revised SOP reviewed and approved by the UniSQ AEC Executive.	Approved